

## WHAT IS CLAIMED IS:

- 1                   1.    An isolated or recombinant nucleic acid molecule that comprises a  
2    polynucleotide sequence that encodes a polypeptide selected from the group consisting of:
  - 3                   a)    a polypeptide having lipid A biosynthesis acyltransferase activity,  
4    wherein the polypeptide comprises an amino acid sequence that is at least about 70%  
5    identical to an amino acid sequence encoded by nucleotides 350-1234 (ORF 2a) of the *LOS*  
6    biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
  - 7                   b)    a polypeptide having glycosyltransferase activity, wherein the  
8    polypeptide comprises an amino acid sequence that is at least about 70% identical to an  
9    amino acid sequence encoded by nucleotides 1234-2487 (ORF 3a) of the *LOS* biosynthesis  
10   locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
  - 11                  c)    a polypeptide having glycosyltransferase activity, wherein the  
12   polypeptide comprises an amino acid sequence that is at least about 50 % identical to an  
13   amino acid sequence encoded by nucleotides 2786-3952 (ORF 4a) of the *LOS* biosynthesis  
14   locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1 over a region at least about 100  
15   amino acids in length;
  - 16                  d)    a polypeptide having  $\beta$ 1,4-GalNAc transferase activity, wherein the  
17   GalNAc transferase polypeptide comprises an amino acid sequence that is at least about 77%  
18   identical to an amino acid sequence as set forth in SEQ ID NO:17 over a region at least  
19   about 50 amino acids in length;
  - 20                  e)    a polypeptide having  $\beta$ 1,3-galactosyltransferase activity, wherein  
21   the galactosyltransferase polypeptide comprises an amino acid sequence that is at least about  
22   75% identical to an amino acid sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29  
23   over a region at least about 50 amino acids in length;
  - 24                  f)    a polypeptide having  $\alpha$ 2,3 sialyltransferase activity, wherein the  
25   sialyltransferase polypeptide comprises an amino acid sequence that is at least about 66%  
26   identical over a region at least about 60 amino acids in length to an amino acid sequence as  
27   set forth in one or more of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:10;

28 g) a polypeptide having sialic acid synthase activity, wherein the  
29 polypeptide comprises an amino acid sequence that is at least about 70% identical to an  
30 amino acid sequence encoded by nucleotides 6924-7961 of the *LOS* biosynthesis locus of *C.*  
31 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

32 h) a polypeptide having sialic acid biosynthesis activity, wherein the  
33 polypeptide comprises an amino acid sequence that is at least about 70% identical to an  
34 amino acid sequence encoded by nucleotides 8021-9076 of the *LOS* biosynthesis locus of *C.*  
35 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

36 i) a polypeptide having CMP-sialic acid synthetase activity, wherein  
37 the polypeptide comprises an amino acid sequence that is at least about 65% identical to an  
38 amino acid sequence encoded by nucleotides 9076-9738 of the *LOS* biosynthesis locus of *C.*  
39 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

40 j) a polypeptide having acetyltransferase activity, wherein the  
41 polypeptide comprises an amino acid sequence that is at least about 65% identical to an  
42 amino acid sequence encoded by nucleotides 9729-10559 of the *LOS* biosynthesis locus of  
43 *C. jejuni* strain OH4384 as shown in SEQ ID NO:1; and

44 k) a polypeptide having glycosyltransferase activity, wherein the  
45 polypeptide comprises an amino acid sequence that is at least about 65% identical to an  
46 amino acid sequence encoded by a reverse complement of nucleotides 10557-11366 of the  
47 *LOS* biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1.

1 2. The isolated or recombinant nucleic acid molecule of claim 1, wherein  
2 the nucleic acid comprises a polynucleotide sequence that encodes one or more polypeptides  
3 selected from the group consisting of:

4 a) a sialyltransferase polypeptide that has both an  $\alpha$ 2,3  
5 sialyltransferase activity and an  $\alpha$ 2,8 sialyltransferase activity, wherein the sialyltransferase  
6 polypeptide comprises an amino acid sequence that is at least about 75% identical to an  
7 amino acid sequence as set forth in SEQ ID NO:3 over a region at least about 50 amino acids  
8 in length;

9 b) a GalNAc transferase polypeptide that has a  $\beta$ 1,4-GalNAc  
10 transferase activity, wherein the GalNAc transferase polypeptide comprises an amino acid

11 sequence that is at least about 75% identical to an amino acid sequence as set forth in SEQ  
12 ID NO:17 over a region at least about 50 amino acids in length; and  
13 c) a galactosyltransferase polypeptide that has  $\beta$ 1,3-  
14 galactosyltransferase activity, wherein the galactosyltransferase polypeptide comprises an  
15 amino acid sequence that is at least about 75% identical to an amino acid sequence as set  
16 forth in SEQ ID NO:27 over a region at least about 50 amino acids in length.

1 3. The nucleic acid molecule of claim 1, wherein the sequence  
2 comparisons are performed using a BLASTP Version 2.0 algorithm with a wordlength (W)  
3 of 3, G=11, E=1, and a BLOSUM62 substitution matrix.

1 4. The nucleic acid molecule of claim 1, wherein the region extends the  
2 full length of the amino acid sequence of the polypeptide.

1 5. The nucleic acid molecule of claim 1, wherein:

2 a) the sialyltransferase polypeptide comprises an amino acid sequence  
3 as set forth in SEQ ID NO:3, SEQ ID NO:5 SEQ ID NO:7 or SEQ ID NO:10;

4 b) the GalNAc transferase polypeptide comprises an amino acid  
5 sequence as set forth in SEQ ID NO:17; and

6 c) the galactosyltransferase polypeptide comprises an amino acid  
7 sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29.

1 6. The nucleic acid molecule of claim 5, wherein:

2 a) the polynucleotide sequence that encodes the sialyltransferase  
3 polypeptide is at least about 75% identical to a nucleic acid sequence as set forth in SEQ ID  
4 NO:2, SEQ ID NO:4, or SEQ ID NO:6 over a region at least about 50 nucleotides in length;

5 b) the polynucleotide sequence that encodes the  $\beta$ 1,4-GalNAc  
6 transferase polypeptide is at least about 75% identical to a nucleic acid sequence as set forth  
7 in SEQ ID NO:16 or over a region at least about 50 nucleotides in length; and

8 c) the polynucleotide sequence that encodes the  $\beta$ 1,3-  
9 galactosyltransferase polypeptide is at least about 75% identical to a nucleic acid sequence

10 as set forth in SEQ ID NO:26 or SEQ ID NO:28 over a region at least about 50 nucleotides  
11 in length.

1 7. The nucleic acid molecule of claim 6, wherein the sequence  
2 comparisons are performed using a BLASTN Version 2.0 algorithm with a wordlength (W)  
3 of 11, G=5, E=2, q= -2, and r = 1.

1 8. The nucleic acid molecule of claim 6, wherein:  
2 a) the polynucleotide sequence that encodes the sialyltransferase  
3 polypeptide has a nucleic acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, or  
4 SEQ ID NO:6;  
5 b) the polynucleotide sequence that encodes the GalNAc transferase  
6 polypeptide has a nucleic acid sequence as set forth in SEQ ID NO:16; and  
7 c) the polynucleotide sequence that encodes the galactosyltransferase  
8 polypeptide has a nucleic acid sequence as set forth in SEQ ID NO:26 or SEQ ID NO:28.

1 9. The nucleic acid molecule of claim 5, wherein the sialyltransferase is a  
2 bifunctional sialyltransferase that has both an  $\alpha$ 2,3-sialyltransferase activity and an  $\alpha$ 2,8-  
3 sialyltransferase activity and the polynucleotide sequence that encodes the sialyltransferase  
4 polypeptide is at least about 75% identical to a nucleic acid sequence as set forth in SEQ ID  
5 NO:2, SEQ ID NO:4.

1 10. An expression cassette that comprises a nucleic acid molecule of claim  
2 1.

1 11. An expression vector that comprises the expression cassette of claim 10.

1 12. A host cell that comprises the expression vector of claim 11.

1 13. An isolated or recombinantly produced polypeptide selected from the  
2 group consisting of:

- 3 a) a polypeptide having lipid A biosynthesis acyltransferase activity,  
4 wherein the polypeptide comprises an amino acid sequence that is at least about 70%  
5 identical to an amino acid sequence encoded by nucleotides 350-1234 (ORF 2a) of the *LOS*  
6 biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
- 7 b) a polypeptide having glycosyltransferase activity, wherein the  
8 polypeptide comprises an amino acid sequence that is at least about 70% identical to an  
9 amino acid sequence encoded by nucleotides 1234-2487 (ORF 3a) of the *LOS* biosynthesis  
10 locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1;
- 11 c) a polypeptide having glycosyltransferase activity, wherein the  
12 polypeptide comprises an amino acid sequence that is at least about 50 % identical to an  
13 amino acid sequence encoded by nucleotides 2786-3952 (ORF 4a) of the *LOS* biosynthesis  
14 locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1 over a region at least about 100  
15 amino acids in length;
- 16 d) a polypeptide having  $\beta$ 1,4-GalNAc transferase activity, wherein the  
17 GalNAc transferase polypeptide comprises an amino acid sequence that is at least about 77%  
18 identical to an amino acid sequence as set forth in SEQ ID NO:17 over a region at least  
19 about 50 amino acids in length;
- 20 e) a polypeptide having  $\beta$ 1,3-galactosyltransferase activity, wherein  
21 the galactosyltransferase polypeptide comprises an amino acid sequence that is at least about  
22 75% identical to an amino acid sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29  
23 over a region at least about 50 amino acids in length;
- 24 f) a polypeptide having  $\alpha$ 2,3 sialyltransferase activity, wherein the  
25 sialyltransferase polypeptide comprises an amino acid sequence that is at least about 66%  
26 identical to an amino acid sequence as set forth in SEQ ID NO:3, SEQ ID NO:5, SEQ ID  
27 NO:7 or SEQ ID NO:10 over a region at least about 60 amino acids in length;
- 28 g) a polypeptide having sialic acid synthase activity, wherein the  
29 polypeptide comprises an amino acid sequence that is at least about 70% identical to an  
30 amino acid sequence encoded by nucleotides 6924-7961 of the *LOS* biosynthesis locus of *C.*  
31 *jejuni* strain OH4384 as shown in SEQ ID NO:1;

32 h) a polypeptide having sialic acid biosynthesis activity, wherein the  
33 polypeptide comprises an amino acid sequence that is at least about 70% identical to an  
34 amino acid sequence encoded by nucleotides 8021-9076 of the *LOS* biosynthesis locus of *C.*  
35 *jejuni* strain OH4384 as shown in SEQ ID NO:1;  
36 i) a polypeptide having CMP-sialic acid synthetase activity, wherein  
37 the polypeptide comprises an amino acid sequence that is at least about 65% identical to an  
38 amino acid sequence encoded by nucleotides 9076-9738 of the *LOS* biosynthesis locus of *C.*  
39 *jejuni* strain OH4384 as shown in SEQ ID NO:1;  
40 j) a polypeptide having acetyltransferase activity, wherein the  
41 polypeptide comprises an amino acid sequence that is at least about 65% identical to an  
42 amino acid sequence encoded by nucleotides 9729-10559 of the *LOS* biosynthesis locus of  
43 *C. jejuni* strain OH4384 as shown in SEQ ID NO:1; and  
44 k) a polypeptide having glycosyltransferase activity, wherein the  
45 polypeptide comprises an amino acid sequence that is at least about 65% identical to an  
46 amino acid sequence encoded by a reverse complement of nucleotides 10557-11366 of the  
47 *LOS* biosynthesis locus of *C. jejuni* strain OH4384 as shown in SEQ ID NO:1.

1 14. The isolated or recombinantly produced polypeptide of claim 13,  
2 wherein the polypeptide is recombinantly produced and at least partially purified.

1 15. The isolated or recombinantly produced polypeptide of claim 13,  
2 wherein the polypeptide is expressed by a heterologous host cell.

1 16. The isolated or recombinantly produced polypeptide of claim 15,  
2 wherein the host cell is *E. coli*.

1 17. The isolated or recombinantly produced polypeptide of claim 13,  
2 wherein the polypeptide is a *C. jejuni* serotype O:2 polypeptide.

1                   18. The isolated or recombinantly produced polypeptide of claim 13,  
2 wherein the polypeptide is a sialyltransferase polypeptide according to g) and the  
3 polypeptide is selected from the group consisting of:  
4                   a polypeptide has both an  $\alpha$ 2,3 sialyltransferase activity and an  $\alpha$ 2,8  
5 sialyltransferase activity and comprises an amino acid sequence that is at least 75% identical  
6 to an amino acid sequence of a *cstII* sialyltransferase encoded by ORF 7a of the *LOS*  
7 biosynthesis locus from *C. jejuni* strain OH4384 as set forth in SEQ ID NO:3;  
8                   a polypeptide that has an  $\alpha$ 2,3 sialyltransferase activity and comprises  
9 an amino acid sequence that is at least 75% identical to an amino acid sequence of a *cstII*  
10 sialyltransferase from *C. jejuni* serotype O:10 as set forth in SEQ ID NO:5;  
11                   a polypeptide that that has an  $\alpha$ 2,3 sialyltransferase activity and  
12 comprises an amino acid sequence that is at least 75% identical to an amino acid sequence of  
13 a *cstII* sialyltransferase from *C. jejuni* serotype O:41 as set forth in SEQ ID NO:7; and  
14                   a polypeptide that that has an  $\alpha$ 2,3 sialyltransferase activity and  
15 comprises an amino acid sequence that is at least 75% identical to an amino acid sequence of  
16 a *cstII* sialyltransferase of *C. jejuni* serotype O:2 as set forth in SEQ ID NO:10.

1                   19. The isolated or recombinantly produced sialyltransferase polypeptide of  
2 claim 18, wherein the sialyltransferase polypeptide has an amino acid sequence selected  
3 from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, and SEQ ID  
4 NO:10.

1                   20. The polypeptide of claim 13, wherein:  
2                   a) the sialyltransferase polypeptide of f) has an amino acid sequence  
3 as set forth in SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:10;  
4                   b) the 1,4-GalNAc transferase polypeptide of d) has an amino acid  
5 sequence as set forth in SEQ ID NO:17; and  
6                   c) the  $\beta$ 1,3-galactosyltransferase polypeptide of e) has an amino acid  
7 sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29.

- 1                   21. A reaction mixture for synthesis of a sialylated oligosaccharide, the  
2 reaction mixture comprising a sialyltransferase polypeptide which has both an  $\alpha$ 2,3  
3 sialyltransferase activity and an  $\alpha$ 2,8 sialyltransferase activity, a galactosylated acceptor  
4 moiety, and a sialyl-nucleotide sugar;  
5                   wherein the sialyltransferase transfers a first sialic acid residue from the  
6 sialyl-nucleotide sugar to the galactosylated acceptor moiety in an  $\alpha$ 2,3 linkage, and further  
7 transfers a second sialic acid residue to the first sialic acid residue in an  $\alpha$ 2,8 linkage.
- 1                   22. The reaction mixture of claim 21, wherein the sialyl-nucleotide sugar is  
2 CMP-sialic acid.
- 1                   23. The reaction mixture of claim 21, wherein the sialyltransferase  
2 polypeptide has an amino acid sequence that is at least about 75% identical to an amino acid  
3 sequence as set forth in SEQ ID NO:3 over a region at least about 50 amino acids in length.
- 1                   24. The reaction mixture of claim 23, wherein the sialyltransferase  
2 polypeptide has an amino acid sequence as set forth in SEQ ID NO:3.
- 1                   25. The reaction mixture of claim 21, wherein the galactosylated acceptor  
2 comprises a compound having the formula  $\text{Gal}\beta 1,4\text{-R}$  or  $\text{Gal}\beta 1,3\text{-R}$ , wherein R is selected  
3 from the group consisting of H, a saccharide, oligosaccharide, or an aglycone group having  
4 at least one carbohydrate atom.
- 1                   26. The reaction mixture of claim 21, wherein the galactosylated acceptor is  
2 attached to a protein, lipid, or proteoglycan.
- 1                   27. The reaction mixture of claim 21, wherein the sialylated oligosaccharide  
2 is a ganglioside, a ganglioside mimic, or a carbohydrate portion of a ganglioside.



1                   28. The reaction mixture of claim 21, wherein the sialylated oligosaccharide  
2 is a lysoganglioside, a lysoganglioside mimic, or a carbohydrate portion of a  
3 lysoganglioside.

1                   29. The reaction mixture of claim 27, wherein the galactosylated acceptor  
2 moiety comprises a compound having a formula selected from the group consisting of  
3 Gal4Glc-R<sup>1</sup> and Gal3GalNAc-R<sup>2</sup>; wherein R<sup>1</sup> is selected from the group consisting of  
4 ceramide or other glycolipid, and R<sup>2</sup> is selected from the group consisting of Gal4GlcCer,  
5 (Neu5Ac3)Gal4GlcCer, and (Neu5Ac8Neu5c3)Gal4GlcCer.

1                   30. The reaction mixture of claim 29, wherein the galactosylated acceptor is  
2 selected from the group consisting of Gal4GlcCer, Gal3GalNAc4(Neu5Ac3)Gal4GlcCer,  
3 and Gal3GalNAc4(Neu5Ac8Neu5c3)Gal4GlcCer.

1                   31. The reaction mixture of claim 21, wherein the galactosylated acceptor is  
2 formed by contacting an acceptor saccharide with UDP-Gal and a galactosyltransferase  
3 polypeptide, wherein the galactosyltransferase polypeptide transfers the Gal residue from the  
4 UDP-Gal to the acceptor.

1                   32. The reaction mixture of claim 31, wherein the galactosyltransferase  
2 polypeptide has  $\beta$ 1,3-galactosyltransferase activity and has an amino acid sequence that is at  
3 least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:27 or SEQ  
4 ID NO:29 over a region at least about 50 amino acids in length.

1                   33. The reaction mixture of claim 32, wherein the galactosyltransferase has  
2 an amino acid sequence as set forth in SEQ ID NO:27 or SEQ ID NO:29.

1                   34. The reaction mixture of claim 31, wherein the acceptor saccharide  
2 comprises a terminal GalNAc residue.

1                   35. The reaction mixture of claim 34, wherein the acceptor saccharide for  
2 the galactosyltransferase is formed by contacting an acceptor for a GalNAc transferase with  
3 UDP-GalNAc and a GalNAc transferase polypeptide, wherein the GalNAc transferase  
4 polypeptide transfers the GalNAc residue from the UDP-GalNAc to the acceptor for the  
5 GalNAc transferase.

1                   36. The reaction mixture of claim 35, wherein the GalNAc transferase  
2 polypeptide has a  $\beta$ 1,4-GalNAc transferase activity and has an amino acid sequence that is at  
3 least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:17 over a  
4 region at least about 50 amino acids in length.

1                   37. The reaction mixture of claim 29, wherein the GalNAc transferase  
2 polypeptide has an amino acid sequence as set forth in SEQ ID NO:17.

1                   38. A method for synthesizing a sialylated oligosaccharide, the method  
2 comprising incubating a reaction mixture that comprises a sialyltransferase polypeptide  
3 which has both an  $\alpha$ 2,3 sialyltransferase activity and an  $\alpha$ 2,8 sialyltransferase activity, a  
4 galactosylated acceptor moiety, and a sialyl-nucleotide sugar, under suitable conditions  
5 wherein the sialyltransferase polypeptide transfers a first sialic acid residue from the sialyl-  
6 nucleotide sugar to the galactosylated acceptor moiety in an  $\alpha$ 2,3 linkage, and further  
7 transfers a second sialic acid residue to the first sialic acid residue in an  $\alpha$ 2,8 linkage.

1                   39. The method of claim 38, wherein the sialylated oligosaccharide is a  
2 ganglioside.

1                   40. The method of claim 38, wherein the sialyltransferase polypeptide has  
2 an amino acid sequence that is at least about 75% identical to an amino acid sequence as set  
3 forth in SEQ ID NO:3 over a region at least about 50 amino acids in length.

1                   41. The method of claim 40, wherein the sialyltransferase polypeptide has  
2 an amino acid sequence as set forth in SEQ ID NO:3.

1                   42. The method of claim 38, wherein the sialylated oligosaccharide is a  
2 ganglioside a lysoganglioside, a ganglioside mimic, or a lysoganglioside mimic.